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Correlation between haplotype of apolipoprotein B gene and natural longevity persons in Uygur Nationality

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This paper investigated the correlation between polymorphisms and haplotypes in the apolipoprotein B (*apoB*) gene (SP-I/D, XbaI-RFLP, VNTR) and natural longevity persons among the Uygur people in Xinjiang. For this purpose, 191 healthy Uygur individuals aged above 90 from Hetian area of Xinjiang were recruited, and another 53 persons aged 65—70 from the same nationality, the same region and with the same gender ratio, served as the control group. Genotyping was performed by PCR-SSP, PCR-RFLP and PCR-sequencing methods. Logistic regression analyses revealed that the frequencies of X^+X^+ genotype, M and L alleles and the genetypes composed of M and L were significantly higher in the longevity group than in the control group. In haplotype analyses, we found that, in the long-lived people, the frequency of haplotypes composed of the X+ and M alleles was significantly higher whereas the frequency of haplotypes composed of the X- and S alleles was significantly lower (both $P<0.05$) I than those of their controls. These results indicated that the S allele, SS genotype and X+-S, D-S, D-X+-S haplotypes were the possible adverse factors, whereas the M, L alleles, X+X+, MM, ML, LL genotypes and I-X+-M, X+-M haplotypes were the possibe protective factors for the naturally long-lived Uygur people in China.

apoB gene, haplotype, allele, genotype, natural longevity, Uygur nationality of China

Health and longevity are an eternal theme and the ultimate challenge of life science. Population aging is an increasingly critical problem nowadays and, therefore, it is of great scientific significance and social influence to delay senescence, prolong healthy life-span, shorten disease period and improve the quality of life for aged people. Researches on longevity are involved with human genetics, immunology, genomics, medicine, psychology, nutrition science, etc, but the existence of longevity gene is now universally acknowledged by the academic circles. And with the completion of human genome project and progression of functional genomics, longevity/gene relationship has attracted popular attention in recent years. Current endeavors have mainly concentrated in the relations between longevity and the genes related with lipid metabolism, energy metabolism and immunity regulation. Such factors as cholesterol ester transfer protein (CETP), angiotensin-converting enzyme (ACE), anti-oncogene (P53), interleukin (IL-6, IL-10), insulin-like growth factor (IGF-I) have been widely studied. These genes do not directly influence life-span but, by interacting with environmental elements, they will alter the organism's susceptibility to diseases[1−5].

Apolipoprotein B(apoB) is the main component of very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and it is also the ligand of LDL receptor. It exists in the plasma mainly in two main forms —— apoB48 and apoB100; the former is synthe

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sized exclusively by the gut and the latter by the liver. Apolipoprotein B participates in the assembly and secretion of either chylomicrons from the small intestine or VLDL from the liver, and it plays an important role in maintaining the structural integrity of VLDL and LDL particles. As a vital ligand for the LDL receptor, it mediates the cellular uptake of cholesterol. Lusis et al.[6] first cloned this kind of gene and assigned it to chromosome 2, through filter hybridization with DNA from human/mouse somatic cell hybrids. The gene was assigned to $2p^{23}$-p^{ter} and extended over 43 kb, containing 29 exons and 28 introns . Moreover, the mRNA isolated from the small intestine of monkey was found to contain sequences homologous to the cDNA of apolipoprotein B100, suggesting that the two forms of apoB intestinal (B48) and hepatic (B100) are coded by a single gene. Knott et al.[7] reported the primary structure of apoB: with a molecular mass of 550 kDa, mature apoB100 consists of 4536 amino acid residues, with a very large mRNA extending over 14.1 kb. As a structural protein, apoB100 plays an important role in metabolism, which includes the following five areas: (1) participating in VLDL synthesis, assembly and secretion; (2) being the necessary apolipoprotein for VLDL synthesis and secretion in the liver, which is rich in triglyceride; (3) being a structural protein of VLDL, IDL and LDL, and participating in lipid transit; (4) (80% of LDL is excreted through the receptors), being a necessary ligand for the combination of LDL and receptors; (5) being a necessity for CM synthesis and secretion, and participating in the absorption of exogenous lipid and transport. ApoB100 heparin-combining area may interact with osamine polysaccharide on the aortic wall, which may be closely associated with the fact that ApoB-100 lipoprotein leads to artherosclerosis. So far, 24 polymorphic sites of this gene have been discovered, among which are the insertion/deletion polymorphism of nine base-pairs (I /D) that lead to the addition or deletion of three amino acids in the signal peptide encoded by the first exon; two restriction fragment length polymorphisms (RFLPs), known as *Xba* I and *Eco*R I; an AT-rich hypervariable region 3′ to the gene. All these polymorphisms are related to the levels of total cholesterol (TC), LDL, apoB and triglycerides (TG), and they are dangerous factors for CAD or MI[8−11].

The percentage of centenarians in population of the Xinjiang Hetian Uygur is significantly higher than the average level of the whole country. The Institute of International Natural Medicine listed the district as one of the four longevity districts in the world [12]. By studying this special population we might be able to establish an ideal model for identifying the longevity-related genes and the relations between genetic factors and longevity. We analyzed the relations between the polymorphisms of apolipoprotein B (*apoB*) gene (including SP-I/D, *Xba* I-RFLP, VNTR and haplotype) and the natural longevity persons of the Xinjiang Uygur people.

1 Material and methods

1.1 Subjects

We studied 131 male and 60 female naturally long-lived Uygur people in Hetian of Xinjiang , 191 in total, among them, 52 were over 100 years old and 139 between 90—99. The age of the long–lived old people was determined based on the following five standards:

(1) The roster of centenarians of life-insurance company.

(2) The four generations kin relation as learned through interviewing villages and families one by one.

(3) Their experience of the four great historical events (① the first pandemic plague in 1902; ② the second occurrence of the plague during 1912—1913; ③ the war against Hui nationality; ④ the land reform in 1952).

We concluded their ages on the following hypotheses: Generally, a person had not any memory about those events before 6 years old; he/she could remember any of them after 12 years old; his/her marriage age $\geqslant$15 years old; the age of joining the army $\geqslant$18 years old. In this way, a deduction would be made by contrasting such primary knowledge with the census register in1981, with an error $\leqslant$5 years.

(4) No contradiction in medical records. Fact was verified by consulting different doctors for three times.

(5) The error between actual age and self-reported age $\leqslant$3 years. The reference formula is: actual age = 0.45×(self-reported age) +50.

The control group consisted of 29 male and 24 female dead persons, 53 in total, without kinship. They died naturally 65—70 years old, and, none in their family clan had lived above 70 at least in the recent two generations. We started investigating on this population 7 years ago, collected their blood samples, and confirmed

their cases through many years' follow-up.

This research was approved by the local bioethical committee and every relevant person signed informed consent. Controls were randomly selected and matched in accordance with age, gender, and resident area.

1.2 Experimental methods

1.2.1 Extraction of human genomic DNA. 5 mL of venous blood sample was collected in an EDTA vial for anticoagulation; erythrocytes were broken by means of hypotension; leucocytes were insulated and the genomic DNA was extracted from the leukocytes by standard alcohol phenyl/chloroform methods. The final purity of them was A_{260}:$A_{280} \geqslant 1.8$.

1.2.2 Agents, apparatuses and reaction system. The restrictive enzyme *Xba* I was bought from BioLabs Company, U.K.; Taq polymerase was provided by Shanghai Fuha Biotechnology Co. Ltd; PCR was amplification instrument PTC-100 made in U.S.A. The synthesis of primers and sequencing were completed by Beijing Hudazhongsheng Technology Development Co. Ltd. Each allele or genotype was identified through PCR-sequencing.

The amplification reaction was carried out in a final volume of 25 μL containing 1 μL of genomic DNA, 2 μL of each primer (0.16 μmol/L), 0.25 μL of Taq polymerase (2 U/μL), 2.5 μL of Taq polymerase buffer (10×), 1 μL of dNTP (2 mmol/L), 0.5 μL of DMSO and 15.75 μL of ddH_2O. 50 μL of liquid paraffin was added to the top to prevent evaporation during the process.

1.2.3 I/D polymorphism analysis of signal peptide of 5′ *apoB* gene. A primer pair was designed to amplify the first exon on the *apoB* gene using oligonucleotide 5′CAG CTG GCG ATG GAC CCG CCG A3′ as the 5′ primer and 5′CCA ATG ACA AAA TCC GTG AGG 3′ as the 3′ primer. The PCR process was completed in the following steps: predenaturation at 94℃ for 120 s, denaturation at 94℃ for 30 s, annealing at 65 ℃ for 30 s, and extension at 72℃ for 30 s, totally 30 cycles; eventually extension at 72℃ for 300 s; then 3 μL of the PCR product and mixing of 3 μL loading buffer. The polymorphic alleles differentiated via insertion or deletion of 9 bp were directly visible after 8% polyacrylamide gel electrophoresis (100 V, 8 h) of the PCR products mixed with loading buffer. The polymorphisms of I/D alleles were observed after silver staining.

1.2.4 *Xba* I RFLP typing of *apoB* gene**.** A primer pair was designed to amplify the 26th exon on the *apoB* gene, using oligonucleotide 5′ACC GGC CCT GGC GCC CGC CAG CA 3′ as the 5′ primer and 5′ AGC AGC AAG AGT CCA CCA ATC 3′ as the 3′ primer. The PCR process was carried out in the followings steps: predenaturation at 94℃ for 120 s, denaturation at 94℃ for 30 s, annealing at 56℃ for 30 s, and extension at 72℃ for 30 s, totally 35 cycles; eventual extension at 72℃ for 300 s. Then 10 μL PCR product was digested with 10 U of *Xba*1 at 37℃ for 4 h in water bath. 10 μL digestion product mixed with 2 μL loading buffer was electrophoresed in 5% ethidium bromide-stained 2% agarose gel (100 V, 30 min), and the differences in polymorphic allele could be directly typed under UV .

1.2.5 Polymorphism analysis of 3′ *apoB*-VNTR**.** A primer pair was designed to amplify the 29th exon on the *apoB* gene, using oligonucleotide 5′CAG CTG GCG ATG GAC CCG CCG A 3′ as the 5′ primer and 5′ACC GGC CCT GGC GCC CGC CAG CA 3′ as the 3′ primer. The PCR process was carried out in the following steps: predenaturation at 94℃ for 300 s, denaturation at 94℃ for 60 s, annealing at 54℃ for 60 s and extension at 72℃ for 90 s, totally 30 cycles; eventually extension at 72℃ for 300 s. Then 10 μL PCR product mixed with 2 μL loading buffer was electrophoresed in 5% ethidium bromide-stained 2% agarose gel (100 V, 60 min) and the differences in polymorphic allele could be directly typed under UV .

1.2.6 Statistical analysis. Both data processing and statistical analysis were conducted by using the SAS8.1 program, 2LD program and EH/EH+ program. Hardy-Weinberg equilibrium was assessed by using the χ^2 test and the FINETTI program. Continuous variables are presented as mean ± SD. The correlation of each single polymorphism with the life-span was measured by the χ^2 test and the Fisher's precise probability test. The correlation between genotypes and the life-span was analyzed by stepwise logistic regression and expressed as an odds ratio (OR) with a 95% confidence interval (CI). Haplotype frequencies for various polymorphism combinations were estimated by using the EH/EH+ program. Pairwise linkage disequilibrium coefficients were calculated with the estimated haplotype frequencies using the 2LD program. The extent of disequilibrium was expressed in terms of D. The EH/EH+ program and an omnibus likelihood ratio test were also conducted to

examine the differences in haplotype frequency profiles between the longevities and the controls. All statistical tests were 2-tailed, and $P < 0.05$ was considered statistically significant.

2 Results

2.1 Clinical characteristics

The demographic and clinical data from all individuals are shown in Table 1. Aside from age, there were no statistically significant differences in respect of gender, blood pressure, BMI and clinical biochemical indications between the longevities and the controls.

Table 1 Comparison of clinical characteristics between the longevities and the controls

	Longevities(191)	Controls(53)	*P*
Gender, M/F	131/60	29/24	
Age(year)	97±3	67±3	<0.05
SBP(mmHg)	116.23±9.65	116.82±8.31	NS
DBP(mmHg)	77.48±11.13	70.62±5.36	NS
BMI(kg/m$^{2)}$	20.64±2.34	21.29±2.49	NS
TC(mmol/L)	4.81±1.02	4.76±0.97	NS
HDL-C(mmol/L)	1.07±0.25	1.10±0.27	NS
LDL-C(mmol/L)	3.22±0.90	2.95±0.95	NS
ApoA(g/L)	1.28±0.19	1.32±0.17	NS
ApoB(g/L)	1.01±0.27	1.15±0.52	NS
TG(mmol/L)	1.66±0.72	1.81±0.75	NS
Glu(mmol/L)	5.92±2.13	5.71±1.17	NS

The values are mean ± SD. NS, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; ApoA, apolipoprotein A; ApoB, apolipoprotein B; TG, triglyceride; Glu, glucose.

2.2 Correlative analyses

2.2.1 I/D polymorphism of signal peptide of 5′ *apoB* gene. There appeared three genotypes: one 93 bp band of II homozygote; two bands of ID heterozygote, 93 bp and 84 bp, respectively; one 84 bp band of DD homozygote (Figure 1).

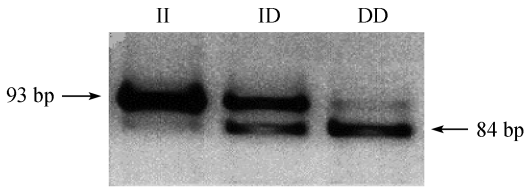

Figure 1 The 5' insertion/deletion polymorphism of the *apoB* gene. "I" represents the *ins* allele and "D" the *del* allele.

2.2.2 *Xba* I RFLP typing of *apoB* gene. Three results were obtained: one 359 bp band of X^-X^- homozygote; three X^+X^- heterozygote bands with a length of 359 bp, 229 bp and 130 bp, respectively; two X^+X^+ homozygote bands with a length of 229 bp and 130 bp, respectively (Figure 2).

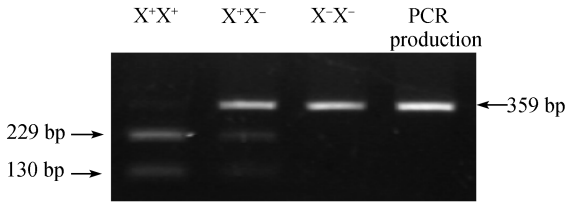

Figure 2 The *Xba* I-RFLP of the *apoB* gene. "X$^+$" refers to *Xba* I restriction site *present* and "X–" the site *absent.*

2.2.3 Polymorphism of 3′ *apoB*-VNTR. The typing was carried out by electrophoretic analysis of PCR amplified DNA fragments as already described[13]. Allele classification was performed by using the same allele ladder in each experiment. The ladder was obtained by PCR amplification of reference DNA samples containing 3′ *apoB*-VNTR alleles of known size. The alleles were nominated according to the number of the repeats in the core sequence motif[14], and classified according to their size and frequency pattern into three groups: small (S), medium (M) and large (L). The S group included alleles with repeats less than 35, M included alleles with 35, 37, 39 repeats, and L included the rest (Figure 3).

2.2.4 The frequency and distribution of alleles and genotypes of three loci and the association analyses. In the 26th exon , the frequency of X^+X^+ genotype was significantly higher in the longevities than that in the controls, but the frequencies of the X^- allele and X^+ allele between the two groups did not show any significant difference. In the stepwise logistic regression analysis, there was a significant increase in OR (OR = 1.296, 95% CI [1.208, 1.390]) in the X^+X^+ genotype carriers in the longevities. In the 29th exon, the repeating times of VNTR were significantly increased in the longevities, apparently higher than that in the controls, i.e. the frequencies of M and L alleles and their frequencies of the genotypes were significantly high. But in the first exon, the frequencies of alleles and genotypes of I/D signal peptide of both groups were not significantly different (Table 2).

2.3 Haplotype analyses

Table 3 shows the results of the haplotype analyses on

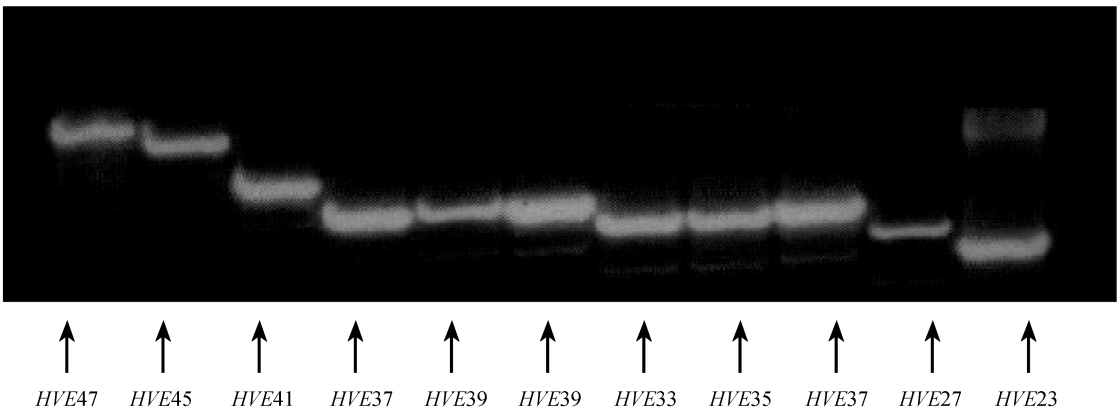

Figure 3 *HVE* means hypervariable elements. Allele nomenclature is based on the number of repeats.

various polymorphism combinations. Of all the four combinations, omnibus haplotype profile tests showed significant elements related to longevity in this population. Univariate analysis on 2-polymorphism combinations showed that the frequencies of haplotypes D-X^+, X^+-M, D-M were significantly higher, while the frequencies of haplotypes X^+-S, D-S were lower in the longevities than that in the controls. Univariate analysis on the 3-polymorphism combination showed that the frequencies of haplotypes D-X^--M, D-X^--L, I-X^+-M were significantly high, while the frequency of haplotypes D-X^+-S was low (P<0.05).

2.4 Linkage disequilibrium analysis on the longevities

There was significant linkage disequilibrium between the I/D and *Xba* I polymorphism (D' =0.31, P<0.001). The linkage disequilibrium of the other two polymorphism combinations (I/D and VNTR, *Xba* I and VNTR) was weak, without any statistically significant difference

Table 2 Comparison of alleles and genotypes frequencies in 3 different regions of *apoB* gene between the longevity group and the control group

Site of gene	Genotype and alleles	*n*(%)	*n*(%)	*P*
5′ signal peptide I/D polymorphisms				
	II	10(5.2)	3(5.7)	
	ID	127(66.5)	33(62.3)	
	DD	54(28.3)	17(32.1)	NS
Frequency	I	38.38	36.79	
of allel (%)	D	61.62	63.21	NS
XbaI-RFLP				
	X^-X^-	120(62.8)	29(54.7)	
	X^+X^-	59(30.9)	24(45.3)	
	X^+X^+	12(6.3)	0(0)	0.03
Frequency	X^-	78.27	77.57	
Of allele (%)	X^+	21.73	22.43	NS
3′VNTR				
	SS	54(28.3)	28(52.8)	
	SM	40(20.9)	9(17.0)	
	SL	3(1.6)	4(7.5)	
	MM	72(37.7)	12(22.6)	
	ML	15(7.9)	0(0)	
	LL	7(3.7)	0(0)	<0.001
Frequency	S	36.53	65.09	
Of allele (%)	M	51.83	31.13	
	L	8.64	3.78	0.0005

NS: Not significant.

Table 3 Estimation of haplotype frequencies in *apoB* gene of the longevity group and the controls

Polymorphism combinations	Haplotype	Overall	Longevities	Controls	χ^2	df	P
SP-I/D & Xba Ⅰ-RFLP	I-X^-	0.319	0.331	0.253	1.554	1	NS[b)]
	I-X^+	0.065	0.059	0.115	2.067	1	NS
	D-X^-	0.461	0.451	0.521	0.981	1	NS
	D-X^+	0.155	0.159	0.111	88.405	1	0.001
XbaI-RFLP & 3-VNRT	Ln(L)[a)]	–412.87	–325.38	–88.73	4.202	3	NS
	X^--S	0.098	0.083	0.152	2.407	1	NS
	X^--M	0.109	0.121	0.064	2.198	1	NS
	X^--L	0.012	0.013	0.011	0.000	1	NS
	X^+-S	0.352	0.312	0.499	7.409	1	0.006
SP-I/D & 3-VNRT	X^+-M	0.366	0.400	0.248	5.128	1	0.024
	X^+-L	0.062	0.071	0.027	1.684	1	NS
	Ln(L)	–591.26	–467.72	–112.24	13.651	5	0.018
	I-S	0.163	0.144	0.231	2.686	1	NS
	I-M	0.192	0.211	0.120	2.940	1	NS
SP-I/D&XbaI-RFLP&3-VNRT	I-L	0.030	0.034	0.017	0.205	1	NS
	D-S	0.288	0.251	0.420	6.486	1	0.011
	D-M	0.284	0.310	0.191	3.840	1	0.050
	D-L	0.043	0.050	0.021	1.332	1	NS
	Ln(L)	–606.51	–476.30	–119.06	13.354	5	0.020
	I-X^--S	0.028	0.024	0.058	2.083	1	NS
	I-X^--M	0.034	0.034	0.046	0.480	1	NS
	I-X^--L	0.000	0.000	0.015	2.000	1	NS
	D-X^--S	0.135	0.120	0.169	1.008	1	NS
	D-X^--M	0.160	0.177	0.078	4.421	1	0.036
	D-X^--L	0.028	0.035	0.003	4.041	1	0.044
	I-X^+-S	0.071	0.059	0.088	0.649	1	NS
	I-X^+-M	0.074	0.087	0.020	4.714	1	0.030
	I-X^+-L	0.012	0.013	0.000	1.005	1	NS
	D-X^+-S	0.217	0.192	0.336	5.776	1	0.016
	D-X^+-M	0.209	0.223	0.168	0.796	1	NS
	D-X^+-L	0.032	0.035	0.020	0.667	1	NS
	Ln(L)	–801.88	–633.25	–158.01	21.636	11	0.027

a) Likelihood ratio statistic for omnibus test in EH program. NS: not significant.

(D' =0.05 and 0.08, respectively).

3 Discussion

All the alleles, genotypes and haplotypes at different areas of the first 26th and 29th exons of *apoB* gene, and their correlations with longevity were studied. Firstly, the frequency of X^+X^+ genotype in the 26th exon was shown to be significantly higher in the longevities than that in the controls and, as revealed by the stepwise logistic regression analysis, and the number of X^+X^+ genotype carriers in the longevities had significant positive correlation with age. Previous researches already indicated that the X^- allele of *apoB* gene was associated with an increase in TC/TG level and the intensive reaction to food among adults, and, at the same time, a lot of researches held that the X^- allele was a main danger to severe atherosclerosis and coronary artery disease[15, 16]. In addition, de Padua Mansur A. et al.[17] reported the probability of atherosclerosis and coronary artery disease was high among X^-X^- genotype carriers. Some studies indicated that apoB-XbaI RFLP polymorphism had some effects on plasma cholesterol, LDLc and ApoB levels. By studying lipoprotein dynamics it was found that these main differences were due to different LDL catabolic rates, and that apoB-XbaI RFLP polymorphism possibly had some influence on the affinity between the lipoprotein and the cell membrane receptors[16]. And, at the same time, *ApoB* might play a core role in the process of lipid assembling and transport. Thus, the hereditary variance of *apoB* gene could explain the correlation between lipid level with atherosclerosis and coronary artery disease. Therefore, the diversity of life-span could be partly explained with different genotype carriers, e.g. a low probability of cardiovascular diseases, such as severe atherosclerosis and

coronary artery disease, might be existent among individual X^+X^+ genotype carriers. However, our study suggested that the two groups exhibited no obvious difference in the frequencies of X^- and X^+ alleles. It probably resulted from the fact that the samples were not sufficient enough, or from the dosage effect of the genotypes.

Secondly, we found the times of VNTR in the 29th exon were obviously higher in the longevities than the controls. In other words, the frequencies of M and L alleles as well as the genotypes composed of them were significantly high. De Benedictis et al.[2] reported that the polymorphisms of 3′ *apoB* VNTR had a correlation with the life-span of Italians in European continent, and the frequency of L allele carried by long-lived people was obviously high. We also found that the frequencies of S allele and SS genotype were obviously lower in the longevities than those in the controls, in accordance with the report of Garasto et al.[18] about Italians. De Benedictis et al.[13] reported that the homozygote of SS genotype was rarely seen in extremely long-lived people, and it would decrease with aging. This fact suggested that L allele was possibly a protective factor for longevity, while the S was a frail gene for longevity. We inferred that the 29th exon of *apoB* gene located at chromosomal telomere and the length of telomere would affect the times of corpuscular cleavage, which further prolonged the life expectancy of cells. Because increasing the repeats number of L allele led to the lengthening of telomere, it might prolong cellular life-span. On the other hand, maybe there was another gene located at this area that had some linkage with longevity.

Friedl et al.[19] and Ye et al.[20] reported independently that the probability of sickening for atheroscolesis and coronary artery disease was very high among the L allele carriers in European Caucasians and Han people in China, while the frequency of S allele was obviously low in the relative patients ill for these diseases, and these diseases were usually considered as risk factors for life-span. The possible reasons for longevity are rather complex. Besides ethnic variance, the difference in the selected samples and gene/environment and gene/gene interaction should also be taken into consideration.

Thirdly, 5′ signal peptide I/D alleles and genotpyes at the first exon did not show any significant difference between the two groups. But in some researches, D allele was considered to be associated with higher TC and TG levels and more intensive food response[21]. The difference of apolipoprotein metabolism after meal could probably be used to explain the correlation of the polymorphism and coronary artery disease. TG level of II homozygote of signal peptide was obviously higher than that of DD homozygote, while the level of ID heterozygote was at the middle of them. Benes et al.[22] reported that I allele was a risk factor for myocardial infarction. Our results revealed that TG and TC levels did not show any significant difference between the two groups, and no evidence could prove that there existed causality between polymorphism of I/D and natural longevity.

Fourthly, the haplotype analysis showed that frequencies of D-X^+, X^+-M, D-M, I-X^+-M, D-X^--M, D-X^--L were obviously higher in the longevities than in the controls, while the frequencies of X^+-S, D-S, D-X^+-S were obviously lower, which were coincident with the alleles and genotypes analyses. Haplotype was composed of different alleles, and so haplotype analysis could provide more information about the effect of gene-gene interaction on phenotype than analysis with a single polymorphic marker. One reason of the effects may owe to the gene synergistic action, such as X^+-M, I-X^+-M; another reason might be that one of the alleles played a leading role, such as D-X^+, D-M, D-S. Otherwise, antagonistic action might exist or some allele might take a dominant place, such as X^+-S, D-X^--M, D-X^--L, D-X^+-S. Because of the complexity of the interactions among genes, these results should be further tested in different races.

Fifthly, the linkage analysis about the three sites and natural longevity showed that there was significant linkage disequilibrium between the I/D and *Xba* I polymorphism sites. The linkage disequilibrium in the other two polymorphism combinations (I/D and VNTR, *Xba* I and VNTR) was weak and not significant. There are possibly two main reasons for the linkage disequilibrium: first, the two different homozygote populations intermixed in short time and exchanged each other at low frequency, which cannot lead to random distribution of the alleles up to now; second, the combination of some alleles at close linkage loci made the carriers possess selective advantage, so it could exist for a long time. Maybe the second explanation was more suitable for longevity but it needs further proof.

To sum up, the results of our research indicate that the S allele, SS genotype and X+-S, D-S, D-X+-S haplotypes are adverse factors to China Uygur naturally long-lived people, whereas M, L allele, X+X+, MM, ML, LL genotypes and I-X+-M, X+-M haplotypes are pro-

tective factors, but the relations and interactions among them remain to be studied. Next, the causes of longevity are extremely complex and a systematical interdisciplinary study is in need; however, by studying the interactions between gene and gene, gene and surrounding environments, we may make clear the mechanism underlying longevity. Since the situation of population aging has become more and more severe nowadays, the topic of longevity will be of momentous scientific and social significance.

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